Parasitology Research (2019) 118:3327–3336
https://doi.org/10.1007/s00436-019-06533-1

FISH PARASITOLOGY - ORIGINAL PAPER

A new myxozoan species *Henneguya unitaeniata* sp. nov. (Cnidaria: Myxosporea) on gills of *Hoplerythrinus unitaeniatus* from Mato Grosso State, Brazil

Letícia Pereira Úngari[1] · Diego Henrique Mirandola Dias Vieira[1] · Reinaldo José da Silva[1] · André Luiz Quagliatto Santos[2] · Rodney Kozlowiski de Azevedo[3] · Lucia Helena O'Dwyer[1]

Received: 15 May 2019 / Accepted: 28 October 2019 / Published online: 15November 2019

Abstract
On the basis of morphological and molecular analyses, a new myxozoan parasite is described from the gills of the fish *Hoplerythrinus unitaeniatus*, collected in the municipality of Nova Xavantina, Mato Grosso State, Brazil. Plasmodia of *Henneguya unitaeniata* sp. nov. were oval and whitish and were found surrounded by collagen fibers forming plasmodia wall between gill filaments on the gill arch. The spores were ellipsoidal with two similar polar capsules. Morphometric analysis showed a total spore mean length of 23.8 ± 1.5 μm, spore body mean length of 14.5 ± 0.7 μm, caudal appendage mean length of 10.3 ± 1.4 μm, thickness mean length of 4.3 ± 0.3 μm, polar capsule mean length of 4.2 ± 0.5 μm, polar capsule mean width of 1.8 ± 0.3 μm, spore mean width of 4.8 ± 0.4 μm, and 4–5 polar filament coils. Phylogenetic analysis showed *Henneguya unitaeniata* sp. nov. as a basal species in a subclade formed by myxozoans that parasitize bryconid fishes.

Keywords Erythrinidae · Histopathology · Myxobolidae · Phylogeny · SSU rDNA

Introduction

The Neotropical region has approximately 8000 species of fishes, which is considered the greatest freshwater fish fauna of the world. Brazil has a large and dense hydrographic network distributed in eight major basins, with a considerable diversity of fish species (Graça and Pavanelli 2007). The Erythrinidae is a small family of Characiformes, represented by three genera: *Erythrinus* Scopoli 1777, *Hoplerythrinus* Gill 1896, and *Hoplias* Gill 1903. *Hoplerythrinus* includes only three species: *H. cinereus* Gill 1858, *H. gronovii* Valenciennes 1847, and *H. unitaeniatus* Agassiz 1829.

Hoplerythrinus unitaeniatus (popularly known as jeju or aimara) is distributed in South and Central America. This species is sedentary and occurs in several types of fluvial and lacustrine environments, especially in shallow water and near submerged or marginal vegetation. The economic importance of jeju fish is related to subsistence fishing, being used as live bait for commercial fishing. Adults of this species are piscivorous, but juveniles also feed on plankton, crustaceans, insects, and seeds (Santos et al. 2006; Soares et al. 2011; Alcântara and Tavares-Dias 2015). Furthermore, this fish species is able to make terrestrial incursions seeking to reach adjacent wetlands (water puddles), which is made possible by facultative aerial respiration using a broadly vascularized and spongy swim bladder (Jucá-Chagas 2004; Mariano et al. 2009). Studies on *H. unitaeniatus* parasite fauna comprise species of Protozoa, Isopoda, Monogenea, Cestoda, Nematoda, Digenea, and Acanthocephala (Chambrier et al. 1996; Rocha 2011; Thatcher 2006; Alcântara and Tavares-Dias 2015). However, there are no reports of myxozoans infecting this fish species.

Myxozoans are microscopic cnidarian parasites, mainly of fishes, belonging to the Class Myxosporea Bütschili 1881,

Section Editor: Sascha L. Hallett

✉ Letícia Pereira Úngari
letspungari@hotmail.com; leticia.p.ungari@gmail.com

[1] Universidade Estadual Paulista (Unesp), Departamento de Parasitologia, Instituto de Biociências, Distrito de Ribeirão Junior, Botucatu, São Paulo, Brazil

[2] Laboratório de Ensino e Pesquisa em Animais Silvestres, Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil

[3] Centro Universitário CESMAC, Maceió, Alagoas, Brazil

Order Bivalvulida Shulman 1959 (Okamura et al. 2015). The occurrence of these fish parasites has been intensively studied mostly due to their pathogenesis (Feist and Longshaw 2006), which includes reduced respiratory capacity (Adriano et al. 2005), longitudinal compressions of body (Longshaw et al. 2003), intestinal necrosis (Alvarez-Pellitero et al. 2008), degenerative cardiomyopathy (Yokoyama et al. 2005), and significant mortalities in wild and farmed fishes that can lead to economic losses (Naldoni et al. 2009).

More than 2400 species of myxozoans have hitherto been described: about 910 species belong to the genus *Myxobolus* Bütschili 1882 (Lom and Dyková 2006), and about 200 species to the genus *Henneguya* Thélohan, 1892 (Eiras and Adriano 2012). In Brazil, approximately 60 *Myxobolus* species and 55 *Henneguya* species have been described (Vieira et al. 2017; Abrunhosa et al. 2018).

For many years, the taxonomic classification of myxozoans was based on morphological characteristics of spores and plasmodia, host organ, and tissue specificity (Andree et al. 1999). However, since 1990s molecular analysis became an important tool to identify and characterize myxozoans species (Andree et al. 1999; Xiao and Desser 2000; Bartholomew et al. 2008).

During a parasitological survey of *H. unitaeniatus*, plasmodia containing myxospores morphologically consistent with *Henneguya* were observed in the gills. The main goal of this study was to describe a new myxozoan species, *Henneguya unitaeniata* sp. nov., by means of morphological and molecular data. In addition, we provide histological details of the plasmodia, analyzing the pathology caused by the parasite.

Material and methods

Fish collection

In August of 2018, one *H. unitaeniatus* specimen was collected in the Nova Xavantina, Mato Grosso State, Brazil (coordinates 14° 31′ 48.03″ S, 51° 41′ 43.88″ W). The specimen was collected by fishing with bamboo sticks and euthanized by neural pithing immediately after capture. The fish was examined within 24 h of capture, and fresh smears of gills were analyzed in a differential interference contrast microscope (Leica DMLB 5000, Leica Microsystems, Wetzlar, Germany) at × 1000 magnification at the Parasitology Department, São Paulo State University (UNESP), Botucatu. The myxozoans were collected from the gills for further morphological and molecular analyses (Vieira et al. 2018). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed (IBAMA license 60640-1).

Morphological analysis

Morphological measurements of fresh spores followed the recommendations of Lom and Arthur (1989): total spore length, spore length, caudal appendage length, polar capsule length, polar capsule width, and spore width. Digital images of more than 30 spores from three plasmodia of *H. unitaeniatus* gills were taken at × 1000 magnification, under light microscopy with Leica software application suite LAS V3.8 (Leica Microsystems).

For histological analysis, the gills were fixed with Karnovsky's solution, and dehydration was initiated afterwards in increasing concentrations of alcohol (× 3 into 70% for 2 h and 95% for 4 h). The material underwent a resin-alcohol mixture for 12 h. Finally, the tissue was embedded in resin (HistoResin, Leica, Germany). The 3-μm sections were stained with hematoxylin-eosin and then examined under a light microscope.

Molecular analysis

One plasmodium from the gills preserved in absolute ethanol was used for molecular analysis. DNA isolation was carried out in accordance with animal tissue protocol of the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Partial SSU rDNA gene was amplified using a combination of PCR and a nested PCR with general eukaryotic and myxozoan primers (Table 1).

Amplifications were performed on a Peltier 200 Thermocycler (MJ Research, Watertown, MA), with initial denaturation at 94 °C for 3 min, followed by 28 or 35 cycles for primary PCR, and 35 cycles for Nested PCR of 94 °C for 40 s, 55 °C for 40 s, 72 °C of 2 min, and a final extension at 72 °C for 10 min. PCR reactions were performed in a 10-μL total volume, containing 0.625 U GoTaq® Flexi PCR polymerase (Promega, San Luis Obispo, California, USA), 0.2 mM for each dNTP, 0.5 μM from each primer, and 10 ng of template DNA. Nested PCR was performed in a 25-μL volume and with 150 ng of PCR product as template.

The products of amplification were subjected to electrophoresis at 80 V in a 1.5% agarose gel, stained with Gel Red, and observed using an ultraviolet transilluminator. The products of interest were purified by adding 2 μL of ExoSAP-IT® (Affymetrix, Santa Clara, CA, USA) to 5 μL of each PCR product according to the manufacturer's recommendations. Amplicon was then sequenced using PCR and nested PCR primer pairs on a 3500 Genetic Analyzer capillary sequencer (Applied Biosystems) after preparation with a BigDye Terminator Cycle Sequencing Ready Reaction Kit v.3.1 (Applied Biosystems), according to the manufacturer's recommendations.

The consensus sequence was obtained by editing and assembling the sequences (forward and reverse) using

Table 1 SSU rDNA primers used in this study, sequence, amplicon size, PCR rounds, and reference

Primer	Sequence 5′-3′	Amplicon size (bp)	PCR round	Author
ERIB1	ACCTGGTTGATCCTGCCAG	≈ 1900	1st	Barta et al. (1997)
ERIB10	CTTCCGCAGGTTCACCTACG G			Barta et al. (1997)
MyxGP2F	WTGGATAACCGTGGGAAA	≈ 800	Nested	Kent et al. (1998)
MyxospecR	GGTTCNCDGRGGGMCCAAC			Fiala (2006)
Act1R	AATTTCACCTCTCGCTGCCA	≈ 900	1st (paired with ERIB1)	Hallett and Diamant (2001)
MyxGen4F	GTGCCTTGAATAAATCAGAG	≈ 900	1st (paired with ERIB10)	Diamant et al. (2004)

BioEdit v.7.0.9 (Hall 2011). The sequence from this study was compared with other myxozoans sequences available in GenBank. The newly generated sequence of partial SSU rDNA was aligned using Geneious version 7.1.3 (Kearse et al. 2012) with ClustalW algorithm (Larkin et al. 2007) and default settings with related sequences that appeared on Blast search. The Bayesian inference (BI) analysis was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), Markov Chain Monte Carlo (MCMC) chains were run for 10 million generations and the log likelihood scores plotted. The "burn in" was set to 25%. Phylogenetic trees were generated and edited in FigTree v1.4 (Rambaut 2012). *Myxidium finnmarchicum* (GQ890672) and *Ceratomyxa amazonensis* (KX236169) were used as outgroup. The aligned sequences of *Henneguya* species from fishes were compared using pair-wise distance (p-distance) matrix.

Results

The morphology of the myxospores found on the gills of *H. unitaeniatus* corresponded with the genus *Henneguya* according to Lom and Dyková (2006) (Figs. 1, 2, 3). As a consensus of morphological and molecular data, a new *Henneguya* species is described.

Description

Henneguya unitaeniata sp. nov. (Figs. 1–3)
Phylum Cnidaria Hatsckek, 1888
Subphylum Myxozoa Grassé, 1970
Class Myxosporea Bütschili, 1881
Order Bivalvulida Shulman, 1959
Family Myxobolidae Bütschili, 1882
Genus *Henneguya* Thélohan, 1892
Henneguya unitaeniatus sp. nov.

Plasmodia Oval and whitish, measuring 100 μm–500 μm. Plasmodia were found surrounded by collagen fibers forming the plasmodia wall between gill filaments on gill arch.

Myxospores Ellipsoidal body with two similarly sized elongated polar capsules, two equal caudal processes, and sporoplasm located at posterior pole of spore. Only mature spores with a unique morphological aspect were observed within the plasmodia. All spores observed had caudal processes. In lateral view, the spores body were fusiform, symmetrical, with a thin suture line at the junction of two thin valves. Total spore mean length was 23.8 ± 1.5 μm, spore body mean length of 14.46 ± 0.75 μm, caudal appendage mean length of 10.3 ± 1.4 μm, thickness mean length of 4.3 ± 0.3 μm, and spore mean width of 4.8 ± 0.4 μm. Polar capsules had a mean length of 4.2

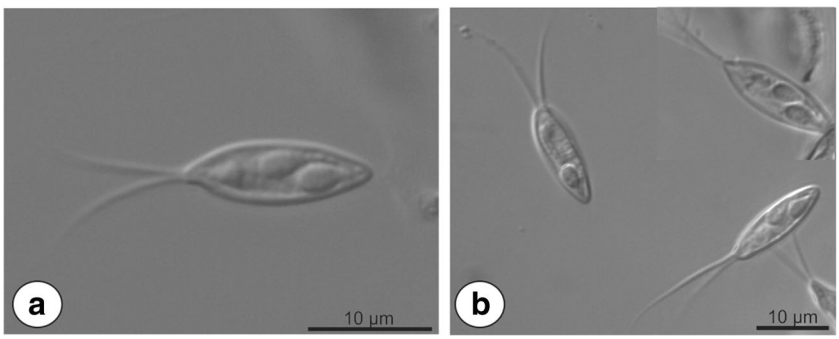

Fig. 1 a–b Light photomicrographs of mature spores of *Henneguya unitaeniata* sp. nov. from the gills of *Hoplerythrinus unitaeniatus.* **a** Mature spore of *Henneguya unitaeniata* sp. nov. **b** Mature spores in frontal and side view. Note detail of the polar filament turning inside the polar capsule (highlight)

Fig. 2 Schematic illustration of *Henneguya unitaeniata* sp. nov. myxospore

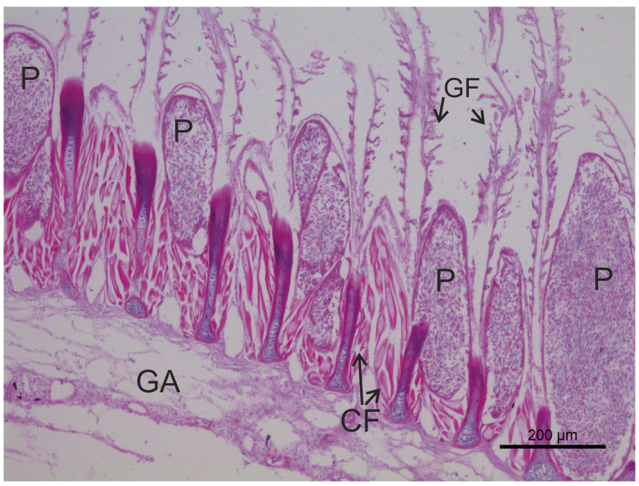

Fig. 3 Photomicrograph of histological section of gills from *Hoplerythrinus unitaeniatus* infected with plasmodia within spores of *Henneguya unitaeniata* sp. nov. Note the gill filaments (GF), collagen fibers (CF), gill filament arch (GA), and the presence of plasmodia (P) in basefilamental position throughout the gill arch

± 0.5 µm and a mean width of 1.8 ± 0.3 µm, with 4–5 polar filament coils (Table 2).

Taxonomic summary

Type-host: Hoplerythrinus unitaeniatus (Actinopterygii: Characiformes: Erythrinidae).

Type-locality: a stream in the municipality of Nova Xavantina, Mato Grosso State, Brazil (14° 31′ 48.03″ S 51° 41′ 43.88″ W).

Site of infection: gills, surrounded by collagen fibers forming the plasmodia wall between gill filaments on gill arch.

Etymology: The epithet specific name (*unitaeniata*) is derived from the name of the host species.

Material deposited: Hapantotype, glycerogelatin glass slides containing myxospores were deposited in the collection of the Nacional Institute of Amazonian Research (INPA), Manaus, Brazil (INPA53, INPA54).

Gene sequence: 1888 bp of SSU rDNA sequence obtained from infected *H. unitaenuatus* gills (GenBank: MN273520).

Morphometric analysis

H. unitaeniata sp. nov. differed from the *Henneguya* species reported in other characiform species from Brazil (Tables 2 and 3). Among the 17 species included in the comparison, nine species have been reported from the gills. The species most resembling *H. unitaeniatus* was *H. rotunda* (Moreira et al. 2014b), which has a total spore length similar to the *Henneguya* found in this study (23.8 ± 1.5 vs 23.6 ± 1.1). However, it is possible to observe a difference when comparing the caudal appendage length (10.3 ± 1.4 vs 16.4 ± 1.2).

When compared with species that infect other organs, the species that most resembled the new species was *H. testicularis*, but differences were observed in total spore length (23.8 ± 1.5 vs 27.5). *Henneguya unitaeniata* sp. nov. was also compared with *H. malabarica*, the only myxozoan species infecting fishes of the Erythrinidae. However, mean size differences were found in all observed parameters. When compared with *Henneguya* species from other geographical regions, the species that most closely resembled *Henneguya unitaeniata* sp. nov. was *H. yunnanensis* Ma, Wang & Cai, 1986. However, *H. yunnanensis* has a body length of 10.4 (8.8–12.8), which is smaller when compared with *H. unitaeniata* sp. nov. (14.5 ± 0.7).

All other species that were compared did not show morphological or morphometric characteristics resembling *H. unitaeniata* sp. nov. At least one morphometric feature, or the number of turns of the polar filament (varying at least 3 turns) of the species abovementioned, differed in comparison with the new species.

Table 2 Morphometric characteristics of the *Henneguya unitaeniata* sp. nov. spores reported infecting *Hoplerythrinus unitaeniatus* gills from Mato Grosso State, Brazil

	SL (μm)	PCL (μm)	CAL (μm)	TSL (μm)	SW (μm)	PCW (μm)	T (μm)
MEAN	14.5	4.2	10.3	23.8	4.8	1.8	4.3
SP	0.7	0.5	1.4	1.5	0.4	0.3	0.3
MAX	15.6	5.4	13.2	25.8	5.7	2.2	4.8
MIN	13.2	3.3	7.4	20.1	4.3	1.3	3.8

SD, standard deviation; *MAX*, maximum measure; *MIN*, minimum measure; *SL*, spore length; *PCL*, polar capsule length; *CAL*, caudal appendage length; *TSL*, total spore length; *SW*, spore width; *PCW*, polar capsule width; *T*, thickness (T)

Molecular and phylogenetic analyses

A fragment of the SSU rDNA gene was amplified (1888-bp long) from spores infecting the gills of *H. unitaeniata* (GenBank: MN273520). The new partial sequence obtained did not match with myxozoans partial sequences available in GenBank, according to the Blast search. The closest species was *Myxobolus batalhensis* (MF361090), which showed 85.1% of similarity and difference of 281 nucleotides. A similarity matrix of the SSU rDNA gene sequences from *Henneguya* species showed that the smallest genetic distance (pair-wise distance) was 0.216 compared with *H. basifilamentalis* (EU732604), and the largest was 0.337 compared with *Henneguya ovata* (KY996557). The isolates used for Bayesian inference (BI) analysis (Table 4) demonstrated two paraphyletic main clades. The new species clustered with *Myxobolus* species from Brazilian salmonid and bryconid fishes (Fig. 4).

Histopathology

Histological analysis of infected gills of *H. unitaeniatus* showed that the plasmodia were of interlamellar type and occurred surrounded by collagen fibers, forming the plasmodia wall between gill filaments on gill arch (Fig. 3). The parasite caused stretching of the epithelium with accentuated deformation, as well as compression of capillary and adjacent tissues. The developmental stages of the parasite occurred in the basifilamental region (Molnár 2002); the plasmodia occupied the entire extent of gill lamellae and produced marked dilation and discreet epithelial hyperplasia. The extensive dilation of infected lamellae caused displacement and deformation of neighboring lamellae. No inflammatory reaction was observed in infected gills.

Discussion

In Brazil, approximately 37 *Henneguya* species have been reported parasitizing gills of fishes (Eiras 2002; Azevedo et al. 2013; Abrunhosa et al. 2018). From those, 21 *Henneguya* species parasitize freshwater characiforms (Casal et al. 2017). However, no previous Brazilian studies have reported infection with a myxozoan parasite in *H. unitaeniatus*.

The morphological characteristics of *H. unitaeniata* sp. nov. are consistent with those of the genus *Henneguya*, including spores with a fusiform to ellipsoidal elongated body, two elongated bifurcated caudal projections, sporoplasm with two nuclei, and two polar capsules (Eiras et al. 2004; Lom and Dyková 2006). The main difference among *Henneguya unitaeniata* sp. nov. and other *Henneguya* species is the position of plasmodia on the gill filament arch. Most of the known species infect either the gill filaments or gill lamellae (Eiras et al. 2005), but some species such as *Henneguya basifilamentalis* Molnár et al. 2006, *Myxobolus basilamellaris* Lom and Molnár 1983, and *Myxobolus lamellobasis* Molnár et al. 2014 develop at the basal part of gill filaments or inside the cartilaginous gill arch (Lom and Molnár 1983; Molnár et al. 2006; Molnár et al. 2014). This development resulted in the formation of large plasmodia at the basal part of gill filaments in all these species, including *H. unitaeniata* sp. nov. Moreover, the overall morphometric analysis of the new species demonstrated that it is different from all other *Henneguya* spp. from Brazilian characiforms (Table 2). *Henneguya rotunda* (Moreira et al. 2014a) is considered the smallest *Henneguya* species described from Brazilian characiforms, but *Henneguya unitaeniata* sp. nov. showed a smaller mean of caudal appendage length.

During the last decade, a large number of myxozoan parasites have been molecularly characterized, including those parasitizing Brazilian fishes (Casal et al. 2017; Vieira et al. 2017; Vieira et al. 2018). The phylogenetic tree obtained for Brazilian myxobolids comprised three clades: two clades of characiform hosts and one clade of siluriform hosts, with some exceptions (Casal et al. 2017; Abrunhosa et al. 2018). The phylogenetic analyses from this study showed a similar result, with *Henneguya unitaeniata* sp. nov. clustering with other species from characiforms.

Myxozoa is known to be a paraphyletic and polyphyletic taxon (Casal et al. 2017; Vieira et al. 2017; Abrunhosa et al.

Table 3 Comparative morphometric data among *Henneguya unitaeniata* sp. nov. with other *Henneguya* species infecting Characiformes fishes from Brazilian ictiofauna

Species	SL (μm)	PCL (μm)	CAL (μm)	TSL (μm)	SW (μm)	PCW (μm)	T (μm)	PFC	Family	Infection site	References
H. unitaeniata sp. nov.	14.5 ± 0.7	4.2 ± 0.5	10.3 ± 1.4	23.8 ± 1.5	4.8 ± 0.4	1.8 ± 0.3	4.3 ± 0.3	4–5	Erythrinidae	Gills	Present study
H. adherens	12.4	3.1	20.5	32.3	5.8	1.2	–	3–4	Acestrorhynchidae	Gills	Azevedo and Matos (1995)
H. malabarica	12.6	3.7	17.1	28.3	4.8	1.8	–	6–7	Erythrinidae	Gills	Azevedo and Matos (1996)
H. testicularis	14.0	9.0	13.5	27.5	6.5	2.0	–	12–13	Characidae	Testis	Azevedo et al. (1997)
H. leporinicola	7.6	3.0	21.8	–	4.2	1.6	3.5 ± 0.1	–	Anostomidae	Gills	Martins et al. (1999)
H. leporini	13–15	5 8	15–18	28–33	5	–	–	–	Anastomidae	Urinary ducts	Nemeczek (1911)
H. travassosi	10.6	3.6	16.7	27.3	4.3	–	–	–	Anostomidae	Muscle	Guimarães and Bergamin (1933)
H. curimata	16.6	6.2	19.1	35.4	6.2	1.2	–	10–11	Curimatidae	Kidney	Azevedo and Matos (2002)
H. friderici	9.6–11.8	4.2–5.9	19.1–28.7	28.7–39.3	4.8–6.6	1.5–2.6	–	7–8	Anostomidae	Several	Casal et al. (2003)
H. schizodon	12–14	5–6	15–17	27–30	3–4	1–1.5	–	8–10	Anastomidae	Kidney	Eiras et al. (2004)
H. pellucida	11.4	4.0	24.1	33.3	4.1	1.6	–	6–7	Serrasalmidae	Gills	Adriano et al. (2005)
H. garavelli	12.0–14.4	4.8–6.0	31.4–35.6	41.6–44.4	3.9–4.1	1.0–1.5	–	8–9	Curimatidae	Gills	Martins and Onaka (2006)
H. cyphocharax	7.7–12.4	4.2–6.3 3.4–5.2	20.8–31.5	29.6–44.4	2.9–6.3	1.5–2.3 1.3–2.2	4.1 ± 0.2	7–9	Curimatidae	Gills	Abdallah et al. (2007)
H. nagelli	12.0 ± 0.5	5.2 ± 0.4 4.9 ± 0.4	22.4 ± 4.0	34.5 ± 4.2	4.9 ± 0.3	1.8 ± 0.2	–	6–8	Curimatidae	Gills	Azevedo et al. (2013)
H. visibilis	10.8 ± 0.6	4.9 ± 0.3	18.0 ± 1.2	26.8 ± 1.1	3.9 ± 0.2	1.4 ± 0.1	3.7 ± 0.5	8–9	Anostomidae	Fins	Moreira et al. (2014b)
H. rotunda	7.1 ± 0.2	3.4 ± 0.2	16.4 ± 1.2	23.6 ± 1.1	5.6 ± 0.2	1.8 ± 0.1	3.7 ± 0.1	6–7	Bryconidae	Gill arch	Moreira et al. (2014a)
H. gilbert	12.0 ± 0.7	5.5 ± 0.3 4.0 ± 0.3	16.8 ± 0.9	27.2 ± 0.8	5.3 ± 0.3	1.3 ± 0.2	3.6 ± 0.2	9–10 7–8	Curimatidae	Gills	Casal et al. (2017)

*SL, spore length; PCL, polar capsule length; CAL, caudal appendage length; TSL, total spore length; SW, spore width; PCW, polar capsule width; T, thickness; PFC, polar filament coils. Measurements are given in micrometers

Table 4 Hosts and GenBank accession numbers for the SSU rDNA sequences of *Myxobolus* spp., *Henneguya* spp. and outgroup used in the phylogenetic analyses (except the sequence from this study)

Species	Host	Accession number (Genbank)
Myxobolus alvarezae	*Aspius aspius*	FJ716097
Myxobolus voremkhai	*Pelteobagrus fulvidraco*	KY229919
Myxobolus physophilus	*Pelteobagrus fulvidraco*	KY421105
Myxobolus prochilodus	*Prochilodus lineatus*	KR528450
Myxobolus curimatae	*Prochilodus costatus*	KP120979
Myxobolus porofilus	*Prochilodus lineatus*	KR528449
Myxobolus batalhensis	*Salminus hilarii*	MF361090
Myxobolus aureus	*Salminus brasiliensis*	KF296348
Myxobolus piraputangae	*Brycon hilarii*	KF296351
Myxobolus umidus	*Brycon hilarii*	KF296350
Myxobolus axelrodi	*Paracheirodon axelrodi*	KU936090
Myxobolus figueirae	*Phractocephalus hemioliopterus*	MG181226
Henneguya mystusia	*Hemibagrus nemurus*	EU732603
Henneguya basifilamentalis	*Hemibagrus nemurus*	EU732604
Myxobolus cf. *colossomatis*	*Piaractus mesopotamicus*	KF597017
Henneguya sp. 4	*Perca fluviatilis*	KY172851
Henneguya eirasi	*Pseudoplatystoma reticulatum*	KF296355
Henneguya laseeae	*Pylodictis olivaris*	KX354352
Henneguya rotunda	*Salminus brasiliensis*	KJ416130
Henneguya ovata	*Trachinotus ovatus*	KY996557
Henneguya lateolabracis	*Lateolabrax* sp.	AB183747
Henneguya cynoscioni	*Cynoscion nebulosus*	JN017203
Henneguya tunisiensis	*Symphodus tinca*	GQ340975
Henneguya mautiraniensis	*Pagrus caeruleostictus*	JQ687060
Myxobolus khaliji	*Acanthopagrus bifasciatus*	KC711053
Henneguya yokoyamai	*Acanthopagrus schlegelii*	AB693053
Myxodium finnmarchicum	*Merlangius merlangus*	GQ890672
Ceratomyxa amazonensis	*Symphysodon discus*	KX236169

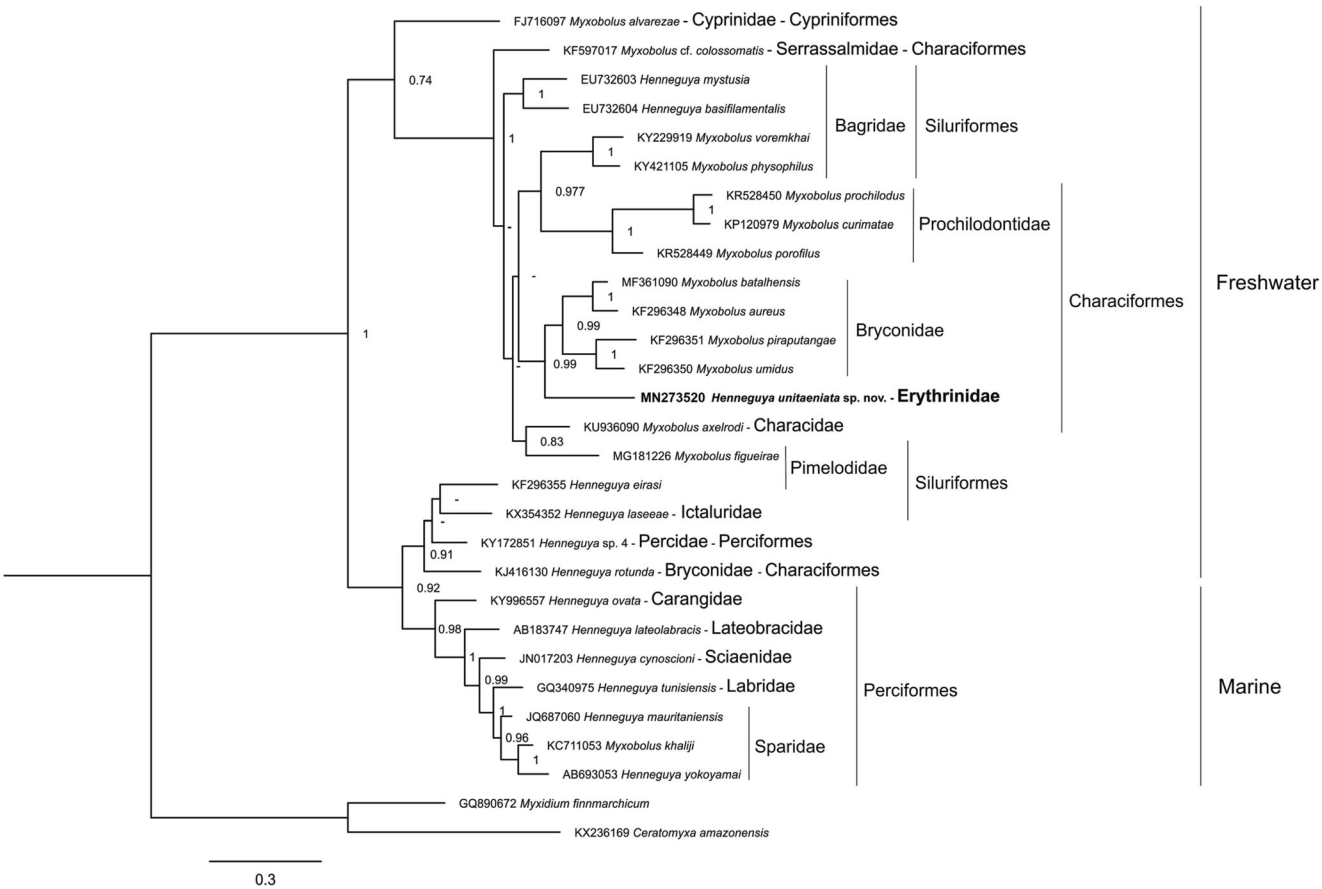

Fig. 4 Bayesian inference (BI) phylogenetic tree based on partial SSU rDNA sequences showing the position of *Henneguya unitaeniata* sp. nov. among other myxozoans from fishes. The scale-bar indicates the distances in substitutions per site. Numbers at nodes represent Bayesian posterior probability gaining more than 0.5 posterior probabilities

2018; Vieira et al. 2019; Li et al. 2019), with affinity between *Henneguya* spp. and *Myxobolus* spp., which was also observed in this study. Although some *Myxobolus* spp. have the capacity to develop spore projections, which is the only morphological difference between the genera *Myxobolus* and *Henneguya*, it is not yet possible to state that they are both the same genus (Li et al., 2019). It is known that habitat, host, and site of infection are considered determining factors to the genetic evolution of myxozoan parasites, being reflected in the taxonomic level of order, family, and genera. Therefore, the arrangement of species belonging to the Myxobolidae is determined by the aquatic environment and host classification, which accounts for the lack of clades containing only *Myxobolus* or *Henneguya* species (Casal et al. 2017; Abrunhosa et al. 2018).

The phylogenetic tree clearly distinguishes *Henneguya unitaeniata* sp. nov from the other *Henneguya* spp. deposited in GenBank. The new species was placed close to the *Myxobolus* species: *M. umidis* (KF296350) from the spleen of *Brycon hilarii*, *M. piraputangae* (KF296851) from the kidney of *B. hilarii*, *M. aureus* (KF296348) from the liver of *Salminus braziliensis*, and *M. batalhensis* (MF361090) from the ovaries of *S. hillarii*, all characiform hosts. This arrangement further corroborates the effectiveness of the SSU rDNA gene for phylogenetic analyses of relationships among myxozoans.

Conclusion

The present study describes *Henneguya unitaeniata* sp. nov., a myxobolid parasite of the characiform host *H. unitaeniatus*, from Mato Grosso State, Brazil, based on morphological, morphometric, and molecular analyses. This study contributes to the study of the biodiversity of myxozoans parasites of fish in Brazil, describing a species that forms plasmodia in an unusual site in the gills. Also, it is the first report of a myxozoan infecting *H. unitaeniatus*.

Acknowledgments We thank the team of the Laboratory for Teaching and Research in Wild Animals (LAPAS-UFU) for their assistance in the fish collection. R.J.S. is supported by FAPESP #2016/50377-1; CNPq #309125/2017-0; CNPq-PROTAX #440496/2015-2.

Funding information We are grateful to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) for financial support.

Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed (IBAMA license 60640-1).

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdallah VD, Azevedo RK, Luke JL, Bonfim TCB (2007) Two new species of Henneguya Thélohan, 1892 (Myxozoa, Myxobolidae), parasitic on the gills of Hoplosternum littorale (Callichthyidae) and Cyphocharax gilbert (Curimatidae) from the Guandu River, State of Rio de Janeiro, Brazil. Parasitol Latinoam 62:35-41

Abrunhosa J, Sindeaux-Neto JL, Hamoy I, Matos ER (2018) A new species of Myxosporea, *Henneguya quelen*, from silver catfish *Rhamdia quelen* (Siluriforme: Pimelodidae) in Amazonian region. Parasitol Res 117:3809–3820. https://doi.org/10.1590/s1984-29612017067

Adriano EA, Arana S, Cordeiro NS (2005) An ultrastructural and histophatological study of *Henneguya pellucida* n. sp. (Myxosporea: Myxobolidae) infecting *Piaractus mesopotamicus* (Characidae) cultivated in Brazil. Parasite 12:221–227. https://doi.org/10.1051/parasite/2005123221

Alcântara NM, Tavares-Dias M (2015) Structure of the parasites communities in two Erythrinidae fish from Amazon river system (Brazil). Braz J Vet Parasitol 24:189–190. https://doi.org/10.1590/S1984-29612015039

Alvarez-Pellitero P, Palenzuela O, Sitjà-Bobadilla A (2008) Histopathology and cellular response in *Enteromyxum leei* (Myxozoa) infections of *Diplodus puntazzo* (Teleostei). Parasitol Int 57:110–120. https://doi.org/10.1016/j.parint.2007.09.004

Andree KB, Székely C, Molnár K, Gresoviac SJ, Hedrick RP (1999) Relationships among members of the genus *Myxobolus* (Myxozoa : Bilvalvidae) based on small subunit ribosomal DNA sequences. J Parasitol 85:68–74. https://doi.org/10.2307/3285702

Azevedo C, Matos E (1995) *Henneguya adherens* sp. n. (Myxozoa, Myxosporea), parasite of the Amazonian fish, *Acestrorhyncus falcatus*. J Eukaryot Microbiol 42:515–518

Azevedo C, Matos E (1996) *Henneguya malabarica* sp. nov. (Myxozoa, Myxobolidae) in the Amazonian fish *Hoplias malabaricus*. Parasitol Res 82:222–224

Azevedo C, Matos E (2002) Fine structure of the myxosporean *Henneguya curimata* n. sp., parasite of the Amazonian fish, *Curimata inormata* (Teleostei, Curimatidae). J Eukaryot Microbiol 49:197–200

Azevedo C, Corral L, Matos E (1997) Light and ultrastructural data on *Henneguya testicularis* n. sp. (Myxozoa, Myxobolidae), a parasire from the testis of the Amazonian fish *Moenkhausia oligolepis*. Syst Parasitol 37:111–114

Azevedo RK, Addallah VD, Paes JVK, Silva RJ, Matos P, Velasco M, Matos E (2013) *Henneguya nagelli* n. sp. (Myxozoa: Myxobolidae) in *Cyphocharax nagelli* (Steindachner, 1881) (Teleostei: Characiformes: Curimatidae) from the Peixe's River, São Paulo State, Brazil. Parasitol Res 112:3601–3605

Barta JR, Martin DS, Liberator PA, Dashkevicz M, Anderson JW, Feighner SD, Elbrecht A, Perkins-Barrow A, Jenkins MC, Danforth HD, Ruff MD, Profous-Juchelka H (1997) Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J Parasitol 83:262. https://doi.org/10.2307/3284453

Bartholomew JL, Atkinson SD, Hallett SL, Lowenstine LJ, Garner MM, Gardiner CH, Rideout BA, Keel MK, Brown JD (2008) Myxozoan parasitism in waterfowl. Int J Parasitol 38:1199–1207. https://doi.org/10.1016/j.ijpara.2008.01.008

Casal G, Matos E, Azevedo C (2003) Light and ultrastructural aspects of *Henneguya striolata* sp. nov. (Myxozoa, Myxosporea), a parasite of the Amazonian fish *Serrasalmus striolatus*. Parasitol Res 83:93–95

Casal G, Clemente SCS, Leila L, Rocha S, Felizardo N, Oliveira E, Al-Quraishy S, Azevedo C (2017) Ultrastructural morphology and phylogeny of *Henneguya gilbert* s. sp. (Myxozoa) infecting the

teleostean *Cyphocharax gilbert* (Characiformes: Curimatidae) from Brazil. Parasitol Res 116:2747–2756

Chambrier A, Scholz T, Vaucher C (1996) Tapeworms (Cestoda: Proteocephalidae) of *Hoplias malabaricus* (Pisces: Characiformes, Erythrinidae) in Paraguay: description of *Proteocephalus regoi* sp. n., and redescription of *Nomimoscolex matogrossensis*. Folia Parasitol 43:133–140

Diamant A, Whipps CM, Kent ML (2004) A new species of Sphaeromyxa (Myxosporea: Sphaeromyxina: Sphaeromyxidae) in devil firefish, *Pterois miles* (Scorpaenidae), from the northern Red Sea: morphology, ultrastructure and phylogeny. J Parasitol 90:1434–1442. https://doi.org/10.1645/GE-336R

Eiras JC (2002) Synopsis of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa: Myxosporea: Myxobolidae). Syst Parasitol 52:43–54. https://doi.org/10.1023/A:1015016312195

Eiras JC, Adriano EA (2012) A checklist of new species of Henneguya Thélohan, 1892 (Myxozoa: Myxosporea, Myxobolidae) described between 2002 and 2012. Syst Parasitol 83:95–104. https://doi.org/10.1007/s11230-012-9374-7

Eiras JC, Malta JC, Varela A, Pavanelli GC (2004) Henneguya schizodon n. sp. (Myxozoa Myxobolidae), a parasite of the Amazonian teleost fish Schizodon fasciatus (Characiformes, Anostomidae). Parasite 11:169–173. https://doi.org/10.1051/parasite/2004112169

Eiras JC, Molnár K, Lu YS (2005) Synopsis of the genus *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). Syst Parasitol 61:1–46

Feist SW, Longshaw M (2006) Phylum Myxozoa. In: Woo PTK (ed) Fish diseases and disorders. Protozoan and metazoan infections. CABI Publishing, Wallingford, pp 230–296

Fiala I (2006) The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. Int J Parasitol 36:1521–1534. https://doi.org/10.1016/j.ijpara.2006.06.016

Graça W, Pavanelli CS (2007) Peixes de planície de inundação do alto rio Paraná e áreas adjacentes Maringá: EDUEM, 1:241.

Guimarães JRA, Bergamin F (1933) Considerações sobre as ictioepizootias produzidas pelos mixosporídeos do género '*Henneguya*' Thélohan, 1892 - *Henneguya travassoi* sp. n. Rev Indust Animal 10:1151–1156

Hall T (2011) BioEdit: an important software for molecular biology. GERF Bull Bisci 2:60–61

Hallett SL, Diamant A (2001) Ultrastructure and small-subunit ribosomal DNA sequence of *Henneguya lesteri* n. sp. (Myxosporea), a parasite of sand whiting *Sillago analis* (Sillaginidae) from the coast of Queensland, Australia. Dis Aquat Org 46:197–212. https://doi.org/10.3354/dao046197

Jucá-Chagas R (2004) Air breathing of the neotropical fishes *Lepidosiren paradoxa*, *Hoplerythrinus unitaeniatus* and *Hoplesternum littorale* during aquatic hypoxia. Comp Biochem Physiol A 139:49–53. https://doi.org/10.1016/j.cbpb.2004.06.019

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kent ML, Khattra J, Hervio DML, Devlin RH (1998) Ribosomal DNA sequence analysis of isolates of the PKX Myxosporean and their relationship to members of the genus. J Aquat Anim Health 10:12–12

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wilm IM, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Lom J, Arthur JR (1989) A guideline for preparation of species descriptions in Myxosporea. K Dish Dis 12:151–156. https://doi.org/10.1111/j.1365-2761.1989.tb00287.x

Lom J, Dyková L (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol 53:1–36. https://doi.org/10.14411/fp.2006.001

Lom J, Molnár K (1983) *Myxobolus basilamellaris* n. sp. (Myxozoa, Myxosporea) parasite of the gills of common carp (*Cyprinus carpio* L.). Folia Parasitol 30:1–3

Longshaw M, Frear P, Feist SW (2003) *Myxobolus buckei* sp. n.(Myxozoa), a new pathogenic parasite from the spinal column of three cyprinid fishes from the United Kingdom. Folia Parasitol 50:251–262

Mariano WS, Oba ET, Santos LRB, Fernandes MN (2009) Respostas fisiológicas de jeju (*Hoplerythrinus unitaeniatus)* exposto ao ar atmosférico. Rev Bras Saúde Prod Anim 10:210–223

Martins ML, Onaka EM (2006) *Henneguya garavelli* n. sp. And *Myxobolus pecularis* n. sp. (Myxozoa: Myxobolidae) in the gills of *Cyphocharax nagelli* (Osteichthyes: Curimatidae) from Rio de Peixe Reservoir, São José do Rio Pardo, São Paulo. Vet Parasitol 137:253–261

Martins ML, Souza VN, Moraes JRE, Moraes FR (1999) Gill infection of *Leporinus microcephalus* Garavello & Britski, 1988 (Myxozoa: Myxobolidae). Description, histophathology and treatment. Ver Brasil Biol 59:527–534

Molnár K (2002) Site preference of fish myxosporeans in the gill. Dis Aquat Org 48(3):197–207

Molnár K, Székely C, Mohamed K, Shaharom-Harrison F (2006) Myxozoan pathogens in cultured Malaysian fishes I. Myxozoan infections of the sutchi catfish *Pangasius hypophthalmus* in freshwater cage cultures. Dis Aquat Org 68(3):209–218

Molnár K, Székely C, Guti CF, Eszterbauer E (2014) Two new *Myxobolus* spp.(Myxozoa: Myxobolidae) from white bream, *Blicca bjoerkna* (Linnaeus, 1758) developing in basifilamental location of gills. Acta Protozool 53(3):277

Moreira GSA, Adriano EA, Silva MRM, Ceccarelli PS, Maia AAM (2014a) The morphological and molecular characterization of *Henneguya rotunda* n. sp. a parasite of the gill arch and fins of *Salminus brasiliensis* from the Mogi Guaçu River, Brazil. Parasitol Res 113:1703–1711. https://doi.org/10.1007/s00436-014-3815-y

Moreira GSA, Adriano EA, Silva MRM, Ceccarelli PS, Maia AAM (2014b) Morphology and 18S rDNA sequencing identifies *Henneguya visibilis* n sp., a parasite of *Leporinus obtusidens* from Mogi Guaçu River, Brazil. Parasitol Res 113:81–90. https://doi.org/10.1007/s00436-013-3629-3

Naldoni J, Arana S, Maia AAM, Ceccarelli PS, Tavares LER, Borges FA, Pozo CF, Adriano EA (2009) *Henneguya pseudoplatystoma* n. sp. causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: histopathology and ultrastructure. Vet Parasitol 166:52–59. https://doi.org/10.1016/j.vetpar.2009.07.034

Nemeczek A (1911) Beitrage zur Kenntnis der Myxo und Microsporidien der Fische. Arch Protistenkd 22:143–169

Okamura B, Gruhl A, Bartholomew J (2015) Introduction. *In*: B. Okamura, A. Gruhl & J.L. Bartholomew (Eds.), Myxozoan evolution, ecology and development. Springer Int Publ 56:1–20

Rambaut A (2012) FigTree v1.4. Molecular evolution, phylogenetics and epidemiology. http://tree.bio.ed.ac.uk/software/figtree/05/2017. Accessed 24 April 2017.

Rocha CAM (2011) Parasitic helminths of the freshwater Neotropical fish *Hoplias malabaricus* (Characiformes, Erythrinidae) from South America basins. Rev Fish Sci 19(2):150–156. https://doi.org/10.1080/10641262.2011.557752

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Santos GM, Ferreira EJG, Zuanon JAS (2006) *Peixes comerciais de Manaus*. Ibama/AM; ProVárzea, Manaus

Soares MGM, Costa EL, Siqueira-Souza F, Anjos HDB, Yamamoto KC, Freitas CEC (2011) *Peixes de lagos do médio Rio Solimões*, 2nd edn. Instituto Piatam, Manaus

Thatcher VE (2006) Amazon fish parasites, 2nd edn. Pensoft Publishers, Sofia, p 509

Vieira DHMD, Alama-Bermejo G, Bartholomew JL, Abdallah VD, Azevedo RK (2017) Morphological and molecular discription of *Myxobolus batalhensis* n. sp. (Myxozoa, Myxosporea), a liver and ovary parasite of *Salminus hilarii* in Brazil. Parasitol Res 116:3303–3313. https://doi.org/10.1007/s00436-017-5644-2

Vieira DHMD, Tagliavini VP, Abdallah VD, Azevedo RK (2018) *Myxobolus imparfinis* n. sp. (Myxozoa: Myxosporea), a new gill parasite of *Imparfinis mirini* Haseman (Siluriformes: Heptapteridae) in Brazil. Syst Parasitol 95:309–318. https://doi.org/10.1007/s11230-018-9776-2

Vieira DHMD, Pelegrini LS, Abdallah VD, Azevedo RK (2019) Supplementary studies on *Henneguya guanduensis* (Cnidaria: Myxosporea) infecting gills and intestine of *Hoplosternum littorale* in Brazil: ultrastructural and molecular data. Parasitol Int 70:27–32. https://doi.org/10.1016/j.parint.2019.01.009

Xiao CX, Desser SS (2000) Cladistic analysis of myxozoan species with known alternating life-cycles. Syst Parasitol 46:81–91

Yokoyama H, Itoh N, Tanaka S (2005) *Henneguya pagri* n. sp. (Myxozoa: Myxosporea) causing cardiac henneguyosis in red sea bream, *Pagrus major* (Temminck & Schlegel). J Fish Dis 28(8):479–487. https://doi.org/10.1111/j.1365-2761.2005.00655.x